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Animal African Trypanosomiasis: time to increase focus on clinically relevant parasite and host species

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African Animal Trypanosomiasis, trypanosome, *Trypanosoma congolense*, *Trypanosoma vivax*, livestock, bovine, immunology

Abstract

Animal African trypanosomiasis (AAT), caused by *Trypanosoma congolense* and *Trypanosoma vivax*, remains one of the most important livestock diseases in sub-Saharan Africa, particularly affecting cattle. Despite this, our detailed knowledge largely stems from the human pathogen *T. brucei* and mouse experimental models. In the post-genomic era the genotypic and phenotypic differences between the AAT-relevant species of parasite or host and their ‘model organism’ counterparts are increasingly apparent. We aim to outline the timeliness and advantages of increasing the research focus on both the clinically relevant parasite and host species – improved tools and resources for both have been developed in recent years. We propose that this shift of emphasis will improve our ability to efficiently develop tools to combat AAT.

Animal African trypanosomiasis – Time to switch models to improve translation of basic research to potential interventions

While human African trypanosomiasis (HAT) has reached the point where eradication is being discussed[1, 2], animal African trypanosomiasis (AAT) remains one of the most significant infectious disease threats to sub-Saharan livestock [3](Figure 1). Although recently there has been a slowly increasing effort to re-focus research on the main causative agents of AAT, *Trypanosoma congolense* and *Trypanosoma vivax*, our specific knowledge of the biology of these pathogens is dramatically outweighed by that for *Trypanosoma brucei*, variants of which cause HAT. Additionally, information on the host response, particularly immunological processes, to these two AAT pathogens in the economically and clinically relevant host – cattle – is scanty compared to the data generated using mouse models (there is a lack of data overall relating to *T. vivax* as most *T. vivax* strains do not grow in mice).

In this article we outline the timeliness and benefits of increasing the research emphasis on both the clinically relevant parasites and host species – recent research developments have resulted in significantly improved tools and resources. We contend that an increased emphasis on furthering our understanding through the use of experimental models that incorporate both *T. congolense*, *T. vivax* and the bovine host will result in more efficient development of useful tools to combat AAT.

AAT – one disease, multiple causative agents

AAT is often treated as a single ‘disease’ but one of several factors in the variation in clinical presentation is that AAT is caused by multiple species and strains of trypanosomes, and often mixed infections. While the most economically important are *T. congolense* and *T. vivax*, *T. b. evansi* is a significant pathogen in cattle, and *T. brucei* s.l. is found in cattle, although it probably has a minor role in pathogenesis. Additionally, within the parasite species, genetic variation results in different clinical outcomes and relevance to disease in cattle, exemplified by greater pathogenicity of *T. b. evansi* compared with *T. b. brucei*, and of *T. congolense* Savannah compared with *T. congolense* Forest or Kilifi (reviewed in [3, 4]). Indeed, there is a requirement for furthering our

understanding of how this complex of species and strains affects AAT disease spectrum and epidemiology - an improved molecular systematics, particularly of *T. congolense* and *T. vivax*, would greatly help to resolve this. While classically thought of as solely an African disease, *T. b. evansi* and *T. vivax* have adapted to mechanical transmission and by this means have spread beyond the tsetse transmission zone in sub-Saharan Africa to become established pathogens affecting the livestock industries of Asia (*T. b. evansi*) and South America (*T. vivax* and *T. b. evansi*)[5, 6].

Antigenic variation and drug uptake are examples of key differences between trypanosome species.

The importance of species-specific parasite knowledge is highlighted by recent examples where fundamental differences have been identified between the three African trypanosome species that indicate significant phenotype differences in traits highly relevant to clinical progression and/or control options. Insight has been accelerated by the successful sequencing of the genomes of *T. congolense* and *T. vivax* (www.tritrypdb.org [7]), and we highlight below two examples where comparative analyses between these species and *T. brucei* [8, 9] has indicated some stark, and perhaps unexpected, differences.

Antigenic variation

African trypanosomes are a paradigmatic organism for antigenic variation [10, 11]. Trypanosomes express this phenotype through the variant surface glycoprotein (VSG), which forms a surface monolayer of homodimers. Antigenic variation works through selective expression of a single copy of antigen, and the active and regular changing of this protein to stay one step ahead of the host adaptive immune response, for which the VSG is highly immunodominant. Trypanosomes have an incredibly elaborate system resulting in an enormous repertoire of antigens (approximately 2000 VSG genes in *T. brucei* [8, 12-14] – dwarfing that of similar pathogens such as *Plasmodium falciparum* that also use antigenic variation [15]). However, almost all of our knowledge on this system was until recently obtained in *T. brucei*. The generation of genome sequence and comparative analysis of *T. congolense* and *T. vivax*, and comparison of the VSG

95 repertoires of these species and *T. brucei*, has revealed some surprising and
96 significant differences [8].

97 *T. brucei* VSGs comprise two types, VSGa and VSGb, as defined by N-terminal
98 domain types (the domains whose epitopes are exposed to the host immune
99 response)[13, 16, 17]. In contrast, *T. congolense* contains no a-type VSGs but only
100 bVSGs, which additionally form two sub-families. Furthermore, in *T. congolense*
101 the bVSG family was further resolved into 15-20 types based on differences in
102 the C-terminal domains (which tether the VSG to the surface membrane and
103 confer structural properties to the VSG protein). All *T. brucei* VSGs share a
104 relatively uniform C-terminal domain that is crucial to the mechanism of genetic
105 recombination between *T. brucei* VSGs; that the situation in *T. congolense* differs
106 so markedly suggests a different mechanism. Therefore, these data indicate
107 significantly greater structural diversity in VSGs in *T. congolense* than *T. brucei*. *T.*
108 *vivax*, which is the most basal branching trypanosome lineage known, was found
109 to possess some VSG types analogous to VSG a and b, but also two further types
110 that did not have orthologues in *T. congolense* or *T. brucei*, suggesting even
111 greater structural diversity than in these two pathogens (however, the identity
112 of these additional types as VSGs requires confirmation). Additionally,
113 phylogenetic analysis of the VSG repertoires revealed evidence for a range of
114 contribution of within-family recombination in generating VSG diversity across
115 the different species, with *T. brucei* displaying evidence of frequent
116 recombination, *T. vivax* relatively little, and *T. congolense* being intermediate.
117 These differences are likely to reflect mechanistic differences in how the species
118 achieve the phenotype of antigenic variation by changing the identity and
119 sequence of the expressed VSG, and importantly, underline that they are very
120 distinct organisms. This may be relevant to potential development of tools, as
121 many of the inferences with respect to antigenic variation and barriers to, for
122 example, vaccine development, are entirely founded upon our knowledge of *T.*
123 *brucei*. It has been known for some time that the VSG monolayer in *T. vivax* is less
124 dense than the VSG coat in *T. brucei* (as indicated by electron micrographs [18]),
125 and transcriptomic studies have demonstrated that VSG expression in *T. vivax*
126 accounts for a significantly smaller proportion of total transcripts than in *T.*
127 *brucei* [19, 20]. Therefore, the role the VSG barrier plays in shielding invariant

antigens (which theoretically could be more conducive to antibody/vaccine targeting) has not been explored in the different species and in *T. vivax* in particular (several *T. vivax*-unique non-VSG protein families have been identified that are predicted to be surface-expressed [19]). Indeed, this canonical notion of the physical VSG barrier in *T. brucei* has been questioned in a recent detailed review [21], highlighting that even in *T. brucei* much dogma remains to be challenged.

Drug resistance

A further example of genetic differences between trypanosome species relating to phenotypes of fundamental importance for disease progression and control is that of transporters of relevance for chemotherapy. Pentamidine and diminazene aceturate are two diamidine drugs used for treating HAT and AAT, respectively. In *T. brucei*, these drugs are transported primarily through the *T. brucei* P2 adenosine transporter 1 (TbAT1 [22]). Diminazene has been the most widely used AAT trypanocide over decades, and as a result resistance is reported [23-25]. Resistant strains of *T. brucei* fail to take up the drug as a result of mutations in TbAT1 [22, 26]. However, when the genome of *T. congolense* was analysed, the putative orthologue of TbAT1 was shown to not be so through both genomic and functional analysis [27] – indeed there is no detectable orthologue in the *T. congolense* genome. Therefore, the main route of diamidine drug uptake, and resistance, must be different in *T. congolense* (and probably in *T. vivax*, given there is also no clear TbAT1 orthologue in the current *T. vivax* genome assembly – see www.tritrypdb.org). These are fundamental differences that will relate directly to drug development initiatives in terms of identifying potential cross-resistance with existing drugs and attempts to predictively identify drug resistance markers by generating resistant lines *in vitro*.

These examples highlight the power of genomic information to fast track our understanding of similarities and differences between trypanosome species, but also underline that *T. brucei* often does not represent a model for *T. congolense* or *T. vivax*. Although we are in the early stages of defining functional relevance of between-species differences, we are entering an era where genomic tools and

resources are available [8, 9, 19], culture of relevant life cycle stages has been reported and, importantly, transfection systems for both organisms are available [28, 29]. Therefore, many of the barriers that previously existed to working with these trypanosome species have been removed or at least minimised. We can now increase our knowledge in the clinically relevant species, which should lead to more successful intervention (e.g. drug) development to combat AAT. For example, information gained in studies involving *T. congolense* and *T. vivax* regarding drug uptake and mechanisms of action, markers of resistance, and cross-resistance to existing compounds, assists drug candidate selection and may extend the useful lifetime of new drugs.

What about the bovine host?

The bovine immune response to trypanosomes is relatively poorly studied, particularly in light of the growing repertoire of tools and reagents that have been developed (see e.g. [30] and Table 1) in recent years. Additionally, several aspects of the bovine immune response have been described recently that are either unique or are significantly different to their human or murine counterparts (e.g. non-conventional T lymphocyte subsets with unique functions, significantly expanded natural killer (NK) cell receptor families, and 'ultralong' antibody CDR3 domains [31-35]). Thus, any potential influence of aspects such as these on trypanosome infections clearly cannot be accurately measured or tested in model organisms such as mice. As well as the continuing development of the repertoire of conventional resources and reagents, and similar to the situation with trypanosomes, we are clearly very much in the post-genomic era for the bovine host (*Bos taurus* and *Bos indicus*), resulting in both the uncovering of key differences between cattle and other species, as well as generation of polyomic datasets that serve as invaluable resources for analysing the bovine immune response [36-38]. It is increasingly clear that gene editing technologies are much more readily applicable to large animals than was previously possible [39], meaning that both in terms of feasibility and cost the alteration of genotype to assess phenotype is now a real option. Much of the work analysing the bovine immune response to trypanosomes was undertaken some time ago (reviewed in [40, 41]). More recently, there have been key insights from bovine genetics

studies (that have not explicitly incorporated immunology) and mouse studies, and we highlight two examples below where application of immunological analysis in cattle may progress our understanding of key phenotypes in AAT.

Trypanotolerance

One aspect that has received much attention is the role of host genetics - some cattle breeds remain infected but do not display the clinical disease of susceptible breeds ('trypanotolerance' [42]). This has been exploited using classical genetics to identify genes and potential pathways involved in successful control of trypanosome infections in the bovine host [43, 44]. While immune response parameters were not explicitly measured phenotypes in these studies, the regions linked to measured phenotypes (parasitaemia, body weight and packed cell volume) contain candidate genes (the alleles of which are responsible for conferring trypanotolerance) whose putative function is in several cases linked to the immune response. In particular, these data indicate that a NK cell receptor gene (*Cd244*), a gene in the Toll-like receptor pathway (*TICAM1*) and genes such as *MAPK* whose effect may influence several immune response pathways, are implicated in controlling trypanotolerance. However, how the products of these genes and pathways influence the bovine immune response and functionally reduce clinical symptoms has not been addressed. To fully validate the involvement of such pathways and genes, it will be essential to analyse immunological function to understand the role that such alleles have in the interaction with trypanosomes.

Much of current knowledge of immune response to trypanosomes has stemmed from the mouse model. This undoubtedly led to significant advances in our understanding, and helped to highlight many of the unique features of trypanosome infections and their interaction with the mammalian immune response. This has included work on the hierarchy of genetic susceptibility to trypanosome infections in mice (in parallel with the bovine trypanotolerance data) that has led to identification of candidate loci and pathways responsible for controlling trypanosome infections in mice [45, 46]. The comparison with cattle trypanotolerance is instructive, as the phenotypes used to assess genotype linkage in the mouse model were necessarily different (survival time in mice

versus multiple pathogenesis phenotypes in cattle) and there was relatively little overlap in identified genes and pathways, probably due to both fundamental organismal differences and differing measured phenotypes. However, there were some interesting overlaps - in particular *Cd244* and the NK cell pathway were implicated in both models [44, 46]. Given the identification of a common process despite the differences in protocol and organism, it is tempting to conclude that NK cells in cattle are worthy of specific attention regarding their role in controlling trypanosome infections. The increasing availability of tools and knowledge [35] to dissect bovine NK cells and their responses will be central to such studies. Humans and mice express distinct NK cell receptor families (KIR and Ly49 (KLRA)) that have functional similarities but are encoded by distinct gene complexes within the genome [47]. Outside of humans and other simian primates, cattle (*B. taurus* & *B. indicus*) are the only species to have an expanded polymorphic KIR gene family [48] and a polymorphic *Ly49* gene [49].

Immunosuppression

A cardinal sign of trypanosomiasis is immunosuppression, and this phenotype is an example where the mouse experimental model has produced interesting and novel insights. Recent studies have demonstrated in the murine model that this is through parasite-driven B cell apoptosis and loss of immunological memory [50-53]. Although the precise mechanism and the parasite ligand that mediates it have not been identified, this phenotype is well defined in mice – the initial work used *T. brucei* but subsequent studies demonstrated a similar effect in *T. congolense* infected mice [54]. It would be interesting and timely to determine if this phenotype occurs in cattle to a similar extent via the same or related mechanisms - there is evidence that specific memory loss occurs in infected cattle [55] but perhaps not to the same degree as in mice. In cattle pre-challenged with irradiated *T. brucei*, then infected with *T. congolense* and subsequently challenged with the same irradiated *T. brucei*, 3 of 5 cattle showed reduced recall response to the *T. brucei* inoculation [55]. Equally pertinent would be to compare whether this phenotype is consistent or varies depending on parasite species in cattle.

The importance and relevance of understanding the bovine immune response to trypanosomes is clear. Understanding the ability of the bovine host to control the parasite has direct implications for potential vaccine development strategies and other anti-disease interventions. The authors wish to emphasise that the purpose of this article is not to minimise what has been achieved or the general utility of mouse models in advancing our understanding (see [56, 57]), but given recent progress in tools and resources we aim to highlight that more emphasis on understanding the bovine model is timely and will reap dividends for enhancing our understanding and control of AAT. At some point during studies of a livestock disease, findings in the murine model need to be validated and translated to the relevant host – our ability to do this meaningfully is now greater than ever.

Concluding Remarks and Future Directions

The genetic and phenotypic differences between *T. brucei*, *T. vivax* and *T. congolense* compel more research focussed on understanding the between-species differences that are pertinent to phenotypes relevant to potential strategies for controlling AAT. Additionally, given recent findings highlighting unique features of bovine immune responses, our understanding of these responses to trypanosomes requires updating, the results of which will undoubtedly feed into defining key aspects of AAT and its control. Moreover, the development of post-genomic resources and tools for both cattle and livestock trypanosome species mean that many barriers to working with these organisms are removed (Figure 2).

However, it cannot be ignored that there are significant challenges involved in moving to the bovine model and limitations that need to be appreciated (Table 1); these largely centre on cost but also the availability of appropriate facilities to run *in vivo* infections on the requisite scale is relatively limited. This places an onus on funders to understand these challenges and to provide the appropriate support for work in cattle – ultimately there is no short cut to generating meaningful progress in the clinically relevant host.

We suggest that research priorities should be directed at applying the tools and resources described in this article to some of the key gaps in our knowledge

relating to both the trypanosome species and the bovine response to them (see Outstanding Questions Box); namely (a) exploiting well characterised phenotypes in *T. brucei* as a platform to analyse key differences in *T. congolense* and *T. vivax* (e.g. antigenic variation, drug transport/resistance), (b) assessing the translation of key phenotypes in the murine model to the bovine host (e.g. B cell apoptosis and immunosuppression), and (c) characterising the role of unique features of the bovine immune response in trypanosomiasis and their interplay with *T. congolense* and *T. vivax*. Advancing our knowledge in these areas will significantly enhance our understanding of trypanosome infection biology in the cow.

Finally, the identification of a holistic, and realistic, approach to controlling AAT will ideally come from integrated studies - using both AAT causative agents and cattle will be more informative in identifying both host and pathogen factors specific to AAT that are amenable to intervention (Figure 2). Therefore, it is timely to increase the research focus on clinically relevant host and trypanosome species for AAT.

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596 TABLE 1. Comparative attributes and challenges of working with either mice or
597 cattle in Trypanosomiasis studies of pathogenesis, pathophysiology and efficacy
598 (e.g. pharmaceutical or vaccine candidates).
599
600

Figure Legends

Figure 1. Distribution of animal African trypanosomiasis caused by *Trypanosoma congolense* and *Trypanosoma vivax*.

Figure 2. Illustrative pipeline for the development of tools against animal African trypanosomiasis (AAT) using an integrated host-parasite approach.

Solid boxes represent current state of knowledge; dashed boxes represent future progress. With the aid of genome sequences key species-specific differences have been identified for both the bovine host and livestock trypanosome species (examples are illustrated in the green and blue boxes, respectively). The exploitation of such findings and increasing the emphasis on research that uses the clinically relevant species of host and parasite will maximise the potential for future tools against AAT – ideally in integrated studies where both parasite and host factors can be identified.

Table 1

Parameter	Mice	Cattle
Cost per animal	Low	High
Ability to scale up numbers & appropriately power experiments	Easy and low cost	Difficult and expensive (Limited facilities worldwide that can incorporate large numbers of infected animals)
Between animal variability	Low – multiple inbred lines available	High – animals are outbred (Also many phenotypes show variation between breeds)
Ability to genetically manipulate (e.g. gene knockout)	Straightforward – many gene knockout lines available.	Currently difficult but prospects improving (e.g. Crispr/Cas9 approaches, but high costs for maintaining lines, long generation time)
Reference genome quality	Very good (Genomes of multiple strains available)	Satisfactory (<i>B. taurus</i> & <i>B. indicus</i> genomes available, annotation patchy)
Predictability of results for use in cattle in field	Low (Useful for basic pathophysiology/immunobiology proof of principle and drug candidate selection after <i>in vitro</i> evaluation)	High
Research tools	Many (Readily available, low cost)	Fewer but rapidly increasing (cellular and molecular tools, reagents & techniques – see [30])
Reagent or Active substance requirement: Quantity & cost	Small (e.g. <1 mg)	Large (e.g. for pharmaceutical, 10-20 g per parasite species)
Animal facilities	Readily available, low cost	Containment and fly-proof facilities usually required (Few and expensive; may require endemic country e.g. <i>T. vivax</i>)
Trypanosome isolates	Mainly laboratory strains (Limited and only one, old strain of <i>T. vivax</i> –Y486)	All can be used (Including recent, drug resistant, field isolates)
Typical efficacy study duration	60 days	100 days
Drug candidate route of administration	S/C or I/P	As intended for final product (e.g. S/C, I/M)
Drug candidate formulation	Usually simple (e.g. DMSO-based for small molecule)	May require formulation development

Figure 1

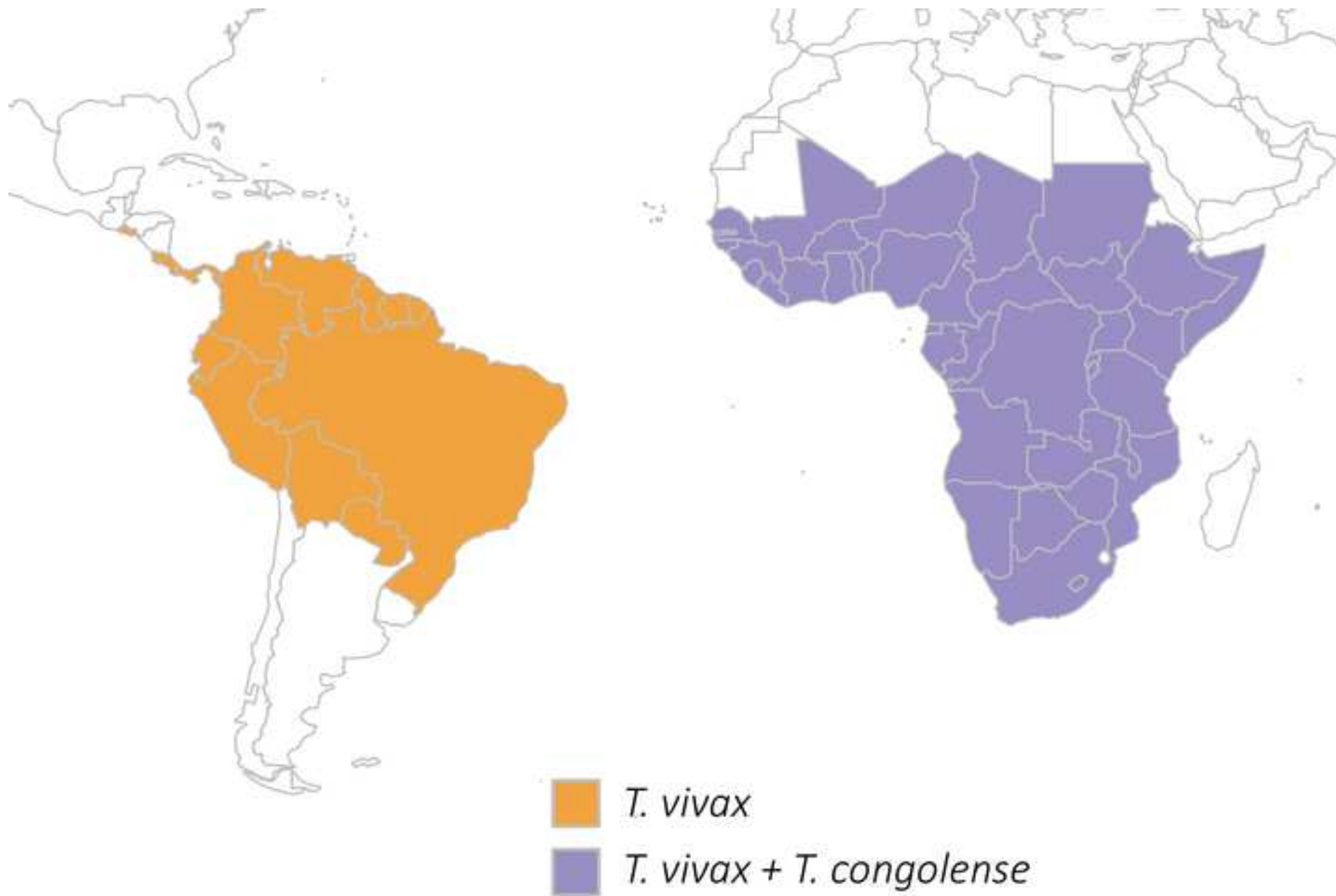
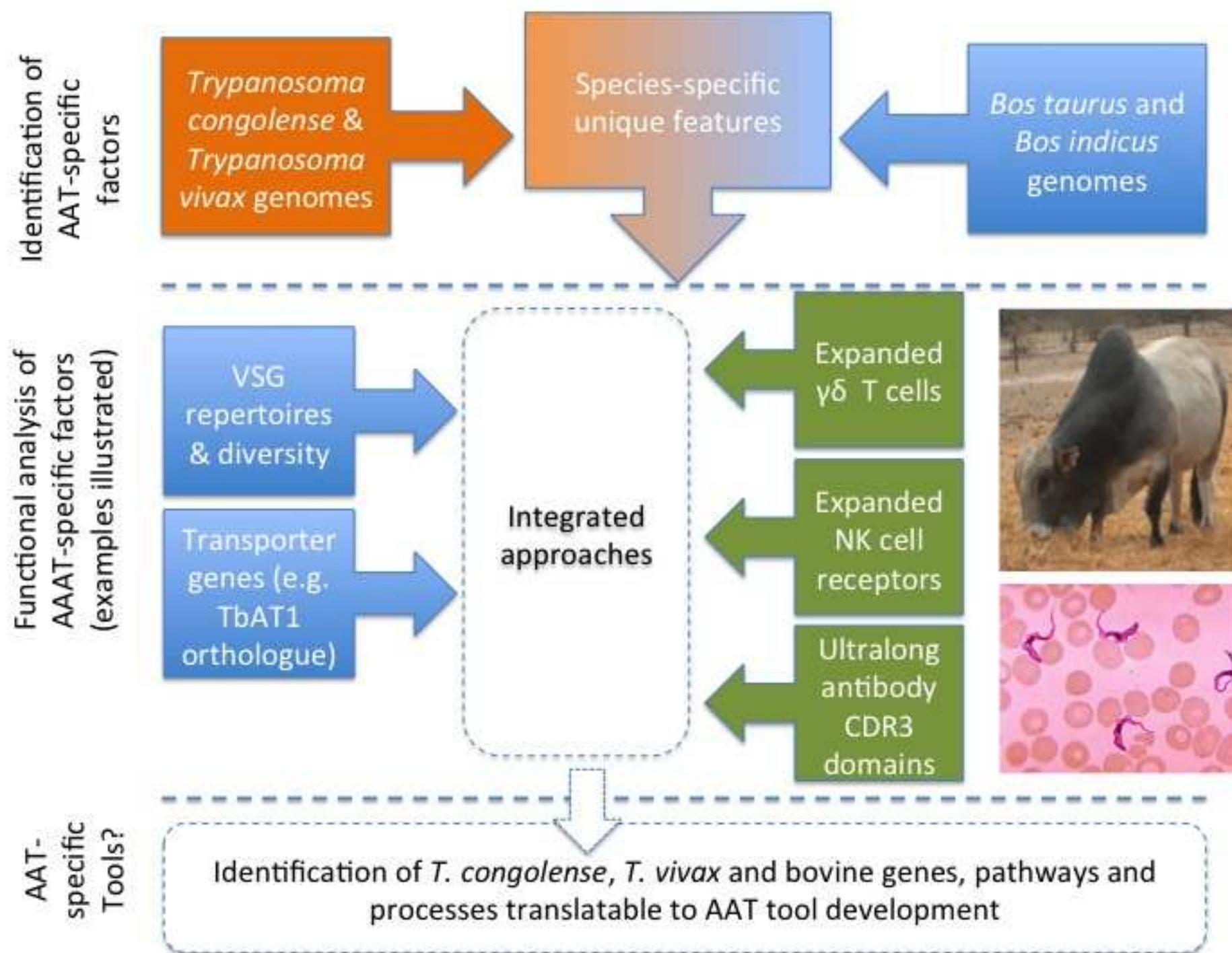


Figure 2



Trends Box

The *T. congolense* & *T. vivax* genomes revealed significant differences in key genes/gene families for relevant phenotypes compared to *T. brucei*.

The variant surface glycoprotein (VSG - confers antigenic variation) repertoires indicate significant divergences in structural diversity and relative role of recombination in generating VSG diversity.

T. congolense lacks an orthologue of the main diamidine transporter in *T. brucei* (TbAT1), meaning the route of drug uptake/resistance is different.

Unique aspects of the bovine immune system have recently been identified, such as increased frequency of $\gamma\delta$ T cell population and ultralong CDR3 domain antibodies.

Natural Killer cells have been implicated in murine & bovine trypanosome susceptibility genetic studies. NK cells in cattle have been recently identified to have a uniquely expanded NK receptor repertoire.

Outstanding Questions Box

- Do the differences in *T. brucei*, *T. congolense* and *T. vivax* VSG repertoire reflect mechanistic differences in how they achieve the phenotype of antigenic variation?
- Can these differences be exploited in either livestock species?
- What are the key differences in transporter gene families of relevance to drug uptake/drug resistance?
- Are there differences in the *T. congolense* and *T. vivax* genome that impact upon mechanism of action/mechanism of resistance for compounds in development?
- What are the implications of differences in the *T. congolense* and *T. vivax* genome for integrated development of drugs that target both pathogens?
- Do any of the unique features of the bovine immune response (e.g. frequency of $\gamma\delta$ T cell population, ultralong CDR3 domain antibodies and expanded NK receptor families) play a role in the immune response to trypanosome infections?
- Can any of the unique features of the bovine immune response be exploited to combat AAT?
- How do the trypanotolerance genes exert their effect in the bovine immune system on trypanosome infections?
- What is the role of cattle NK cells in trypanosome infections?
- Does immunosuppression in cattle trypanosome infections occur via the same mechanism as identified in mice?
- Does the same parasite ligand mediate this effect in mice and cattle, and is it conserved across *T. brucei*, *T. congolense* and *T. vivax*?